

Research Note

Fluorescent Marker for the Detection of Crop and Upper Gastrointestinal Leakage in Poultry Processing Plants¹

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ABSTRACT Previous published research has identified the crop as a source of *Salmonella* and *Campylobacter* contamination for broiler carcasses and reported that broiler crops are 86 times more likely to rupture than ceca during commercial processing. Presently, we evaluated leakage of crop and upper gastrointestinal contents from broilers using a fluorescent marker at commercial processing plants. Broilers were orally gavaged with a fluorescent marker paste (corn meal-fluorescein dye-agar) within 30 min of live hang. Carcasses were collected at several points during processing and were examined for upper gastrointestinal leakage using long-wavelength black light. This survey indicated that 67% of the total broiler carcasses were positive for the marker at the rehang station following head and shank removal. Crops were me-

chanically removed from 61% of the carcasses prior to the cropper, and visual online examination indicated leakage of crop contents following crop removal by the pack puller. Examination of the carcasses prior to the cropper detected the marker in the following regions: neck (50.5% positive), thoracic inlet (69.7% positive), thoracic cavity (35.4% positive), and abdominal cavity (34.3% positive). Immediately prior to chill immersion, 53.2% of the carcasses contained some degree of visually identifiable marker contamination, as follows: neck (41.5% positive), thoracic inlet (45.2% positive), thoracic cavity (26.2% positive), and abdominal cavity (30.2% positive). These results suggest that this fluorescent marker technique may serve as a useful tool for rapid identification of potential changes, which could reduce the incidence of crop rupture and contamination of carcasses at processing.

(Key words: crop, poultry processing, fluorescent marker, carcass contamination)

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INTRODUCTION

New regulatory requirements and consumer demands require a reduction of foodborne pathogens on commercially processed poultry in the United States. Additionally, export restrictions to some countries have required more focused evaluation of pathogen contamination of carcasses.

Carcass contamination increases at the different stages of processing (Lillard, 1988). *Salmonellae* are one of the

primary pathogens associated with foodborne illness because of their ability to colonize the gastrointestinal tract of poultry and other livestock (Turner et al., 1998). After colonization of the gastrointestinal tract, the highest populations of *Salmonella* are found in the cecum, cloaca, ileum, and to a lesser extent the crop (Barrow et al., 1988). Although levels of salmonellae tend to be lower in the crop compared to other sections of the gastrointestinal tract, the crop has been observed to rupture or leak more frequently than the ceca in broilers and to be more frequently contaminated (Hargis et al., 1995). Recently, our laboratories have observed a significant increase in *Salmonella* and *Campylobacter* incidence in the crops of market-age broilers after feed withdrawal (Byrd et al., 1998, Corrier et al., 1999). This increase in the incidence of pathogenic bacteria in the crops of market-age broilers has furthered the concern for identification of critical control points for pathogen reduction within commercial plants. To help reduce bacterial numbers on broiler carcasses, our laboratory developed a method to visually identify specific points that may

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contribute to the contamination of carcasses. The purpose of the present study was to evaluate a fluorescent marker as a means to identify small amounts of leakage from the upper gastrointestinal tract as a potential tool for identifying evisceration changes that could reduce the occurrence of upper gastrointestinal contamination.

MATERIALS AND METHODS

Fluorescent Marker

A mixture of 1 mg fluorescein dye⁴/mL, 20% corn meal, and 2% agar⁵ was used for gavage. The agar (2%) was boiled and mixed with 20% corn meal, and 1 mg fluorescein/mL was added to the mixture while continually stirring. This mixture, subsequently referred to as fluorescent marker, was loaded into a 60-cc catheter syringe⁶ and stored at 4 C.

Experiment 1

One hundred commercial market-age broilers, divided into four groups of 25 birds each, were orally gavaged with 5, 10, 18, or 25 mL of the fluorescent marker approximately 30 min prior to processing. The broilers were hung on every other shackle and slaughtered by the normal processing procedures at a commercial processing plant. Ten carcasses from each group were removed from the table at the manual rehang station, placed in separate plastic bags to prevent cross contamination, and evaluated for visible carcass contamination with the fluorescent marker. The gastrointestinal tract was dissected and evaluated for passage of the marker. Positive results were recorded when high wavelength (365 nm) black light⁷ caused the appearance of strong fluorescence characteristic of the fluorescent marker. No such fluorescence was noted when nontreated control carcasses were examined (data not shown). Fifteen eviscerated carcasses were removed from the processing line immediately prior to the final wash, placed in separate bags, and evaluated for marker contamination under black light. The areas evaluated on the carcasses were total external skin of the carcass, neck skin, thoracic inlet, thoracic cavity, abdominal cavity, and presence or absence of the crop. The parameters evaluated on each carcass were the incidence of contamination (total positive/total evaluated) and given a mean contamination score. The mean contamination score was the average of an assigned numerical score according to the closest approximation to one of the following: 0 = no visible contamination; 1 = trace of marker; 2 = total positive marker area of 3.14 cm² (size of a United States five cent coin); 3 = total positive marker area of 4.91 cm² (size of a United States twenty-five cent coin, or greater).

Experiment 2

From each of four commercial processing complexes, broilers at 6 wk of age underwent feed withdrawal for 8 to 16 h prior to being caught and transported to the processing plant. Ninety broilers from each processing plant were gavaged with 10 mL of fluorescent marker approximately 30 min prior to processing in processing plants in three states of the southern United States for a total of 360 broilers. Broilers were hung on every other shackle and slaughtered by normal processing practices. Broiler weights ranged from 1,134 to 2,268 g (2.5 to 5 lb), depending on the commercial requirements of the individual processing plant. Ten carcasses from each group were removed from the manual rehang table and placed in separate plastic bags to prevent cross contamination. The carcasses were evaluated for carcass contamination with the marker, and the gastrointestinal tract was dissected and evaluated for marker passage as described above. Twenty carcasses were removed from the processing line at each of the following locations: prepack puller, postpack puller (precropper), postcropper, and after the final wash. Each carcass was placed in a separate bag, evaluated for marker contamination under a black light, and scored as described above.

RESULTS AND DISCUSSION

Mead et al. (1994) evaluated nine different sites in a commercial processing plant using a nalidixic-resistant *Escherichia coli*. These researchers found that broiler transport crate cleaning time was an important control point that should be addressed to prevent the spread of pathogenic bacteria. Furthermore, the killing knife and defeathering distributed the marker *E. coli* to two hundred broiler carcasses after the initial inoculation (Mead et al., 1994). Bioluminescence has been used previously to study the recovery of *Salmonella hadar* transformed with a bioluminescent gene obtained from *Photobacterium phosphoreum* (Bautista et al., 1998). Turkey carcasses were contaminated with the bioluminescent *Salmonella* and were monitored during storage of the food product. The autobioluminescent *Salmonella* metabolic activity was suppressed by lactic acid and storage at 5 C (Bautista et al., 1998). These studies required special equipment and the introduction of pathogenic bacteria into a commercial or experimental processing plant. The advantage of the food-grade fluorescent marker used in the present study was that no culturing was required and provided immediate, sensitive, and inexpensive results that may be useful in commercial processing plants.

A high incidence of carcass contamination with the fluorescent marker was observed during processing of broilers gavaged with 5, 10, 18, or 25 mL of the marker approximately 30 min prior to processing (Table 1). Broilers gavaged with the fluorescent marker and removed at the manual rehang station had the highest marker carcass contamination (70%) when given 18 mL compared to broilers given 5 or 25 mL, which resulted in a 30% carcass contami-

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TABLE 1. Incidence of upper intestinal leakage from market age broiler chickens detected by a fluorescein dye marker¹

Location	Volume (mL)	Sample	Number positive/total tested (%)	Average mean score ²	Average mean score for total positive
Rehang table	5	Whole carcass	3/10 (30%)	0.3	1
	5	Neck	2/10 (20%)	0.2	1
	5	Gizzard	9/10 (90%)	NS ³	NS
	10	Whole Carcass	4/10 (40%)	0.3	1
	10	Neck	2/10 (20%)	0.2	2
	10	Gizzard	8/10 (80%)	NS	NS
	18	Whole carcass	7/10 (70%)	1.0	1.43
	18	Neck	6/10 (60%)	1.4	2.33
	18	Gizzard	9/10 (90%)	NS	NS
	25	Whole carcass	3/10 (30%)	0.3	1
	25	Neck	4/10 (40%)	0.7	1.75
	25	Gizzard	8/10 (80%)	NS	NS
Final wash	5	Whole carcass	5/16 (31%)	0.38	1.2
	5	Neck	6/16 (20%)	0.38	1.0
	5	Thoracic cavity	3/16 (19%)	0.25	1.33
	5	Abdominal cavity	6/16 (38%)	0.5	1.33
	10	Whole carcass	9/15 (60%)	0.67	1.11
	10	Neck	13/15 (87%)	1.53	1.83
	10	Thoracic cavity	8/15 (53%)	1.0	1.88
	10	Abdominal cavity	9/15 (60%)	1.27	2.11
	18	Whole carcass	13/15 (87%)	0.3	1.38
	18	Neck	14/15 (93%)	1.87	1.92
	18	Thoracic cavity	11/15 (73%)	1.2	1.70
	18	Abdominal cavity	11/15 (73%)	1.73	2.3
	25	Whole Carcass	8/15 (53%)	1.93	2.23
	25	Neck	9/15 (60%)	2.33	2.60
	25	Thoracic cavity	9/15 (60%)	1.0	1.67
	25	Abdominal cavity	8/15 (53%)	1.20	2.25

¹Data presented are shown as the number of positive samples over the total number of samples evaluated.

²The mean is the average of an assigned numerical score according to the closest approximation of the following: 0 = no visible contamination; 1 = trace of marker; 2 = total positive marker area of 3.14 cm² (size of a United States nickel coin); 3 = total positive marker area of 4.91 cm² (size of a United States quarter dollar coin, or greater).

³NS = not sampled.

nation rate in each case. Similarly, broilers gavaged with 18 mL of the fluorescent marker and removed prior to the final wash had the highest carcass contamination rate (87%) compared to broilers gavaged with 5 mL (31%), 10 mL (60%), or 25 mL (53%) of marker. Ten milliliters of the fluorescent marker was selected for the second experiment because this volume was the least possible volume that resulted in a residual amount of marker in the crop after 30 min of waiting (Figure 1). Also, this 10 mL of the fluorescent marker selected did not significantly ($P < 0.05$) increase the crop weight compared to nongavaged controls in controlled studies in Experiment 1 (data not shown).

In the second experiment, a high incidence of carcass contamination with the fluorescent marker was observed in commercial market-age broilers gavaged with 10 mL of the fluorescent marker approximately 30 min prior to processing (Table 2). The fluorescent marker migrated as low as the gizzard, but no further, in 88% of the carcasses removed from the processing line at the rehang station. Forty of sixty (67%) of the carcasses removed from the rehang station were contaminated, with 32 (53%) showing contamination of the neck skin. The mean contamination score was less than one, indicating a small area of contamination and suggesting that this contamination occurred at the manual or automated transfer station. The incidence

of carcass contamination and mean contamination scores continued to increase during processing and were highest in carcasses removed from the post-cropper location. However, carcasses removed after the final wash had a significantly lower incidence in marker contamination than observed on carcasses removed immediately postcropper, suggesting that a substantial amount of the fluorescent dye was washed away or quenched during washing. Similarly, carcasses removed following final (prechill) wash were less contaminated in the thoracic inlet, thoracic cavity, and abdominal cavity, again suggesting that the fluorescent marker was physically removed or quenched (Table 2).

These results suggest that this fluorescent marker technique may be a cost-effective, simple, and highly visible means of identifying causes of carcass contamination with upper gastrointestinal contents during processing and evaluating procedural or equipment changes for possible improvements. This technique may also be useful for training processing plant personnel with more effective and safe evisceration techniques. Because elimination or reduction in leakage of upper gastrointestinal tract contents during processing will reduce this potential source of pathogen contamination, visual demonstration of fluorescent marker leakage could be used to identify potential critical control points in a rapid and cost-effective manner.

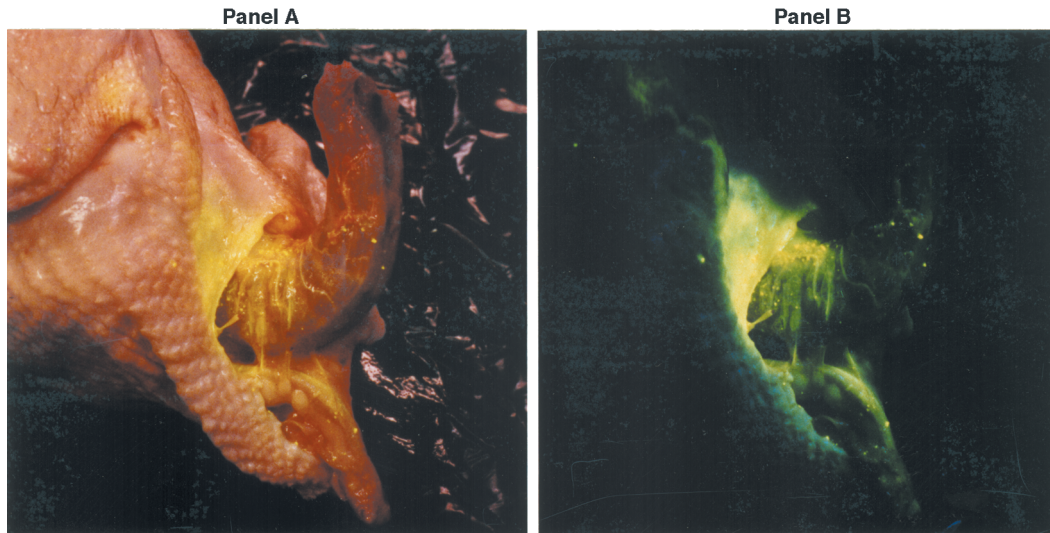


FIGURE 1. Broiler carcasses administered 10 mL of fluorescent marker 30 min prior to processing and removed at manual rehang station (transfer from kill processing line to evisceration processing line). Panel A represents a carcass with a positive result (mean score of 4) recorded when exposed to a high-wavelength (365 nm) black light and normal fluorescent lighting. Panel B represents the same carcass exposed to a high-wavelength blacklight without additional lighting that caused the appearance of strong fluorescence characteristic of the fluorescent marker.

TABLE 2. Incidence of upper intestinal leakage from market age broiler chickens using 10 mL of a fluorescein dye marker (n = 6 plants)¹

Location	Sample	Number positive/total tested (%)	Average mean score ²	Average mean score for total positive
Rehang table	Whole carcass	40/60 (67%)	0.8	1.6
	Neck	32/60 (53%)	0.73	1.24
	Gizzard	53/60 (90%)	NS ³	NS ³
Pack puller	Whole carcass	78/100 (78%)	1.00	1.24
	Neck	52/100 (52%)	0.59	1.04
	Thoracic inlet	70/100 (70%)	1.08	1.41
	Crop presence	100/100 (100%)	NS	NS
	Thoracic cavity	9/100 (9%)	0.13	1.2
	Abdominal cavity	20/100 (20%)	0.26	1.05
Precrop (Postpack puller)	Whole carcass	91/99 (91.9%)	1.21	1.32
	Neck	50/99 (52%)	0.60	1.3
	Thoracic inlet	69/99 (69.7%)	1.22	1.71
	Crop presence	39/99 (39.4%)	NS	NS
	Thoracic cavity	35/99 (35.4%)	0.73	1.44
	Abdominal cavity	34/99 (34.3%)	0.66	1.8
Postcrop	Whole carcass	90/96 (93.8%)	1.22	1.17
	Neck	43/96 (44.8%)	0.58	1.21
	Thoracic inlet	63/96 (65.6%)	1.08	1.56
	Crop presence	3/96 (4.0%)	NS	NS
	Thoracic cavity	32/96 (33.3%)	0.56	1.26
	Abdominal cavity	38/96 (39.6%)	0.66	1.41
Final wash	Whole carcass	132/248 (53.2%)	0.72	1.24
	Neck	103/248 (41.5%)	0.67	1.55
	Thoracic inlet	112/248 (45.2%)	0.89	1.84
	Crop presence	11/248 (4.4%)	NS	NS
	Thoracic cavity	65/248 (26.2%)	0.44	1.43
	Abdominal cavity	75/248 (30.2%)	0.55	1.94

¹Data presented are shown as the number of positive samples over the total number of samples evaluated.

²The mean score is the average of an assigned numerical score according to the closest approximation of the following: 0 = no visible contamination; 1 = trace of marker; 2 = total positive marker area of 3.14 cm² (size of a United States nickel coin); 3 = total positive marker area of 4.91 cm² (size of a United States quarter dollar coin, or greater).

³NS = not sampled.

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